Lentiviral vectors and protocols for creation of stable hESC lines for fluorescent tracking and drug resistance selection of cardiomyocytes.

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Public Summary:

Scientific Abstract:
BACKGROUND: Developmental, physiological and tissue engineering studies critical to the development of successful myocardial regeneration therapies require new ways to effectively visualize and isolate large numbers of fluorescently labeled, functional cardiomyocytes. METHODOLOGY/PRINCIPAL FINDINGS: Here we describe methods for the clonal expansion of engineered hESCs and make available a suite of lentiviral vectors for that combine Blasticidin, Neomycin and Puromycin resistance based drug selection of pure populations of stem cells and cardiomyocytes with ubiquitous or lineage-specific promoters that direct expression of fluorescent proteins to visualize and track cardiomyocytes and their progenitors. The phospho-glycerate kinase (PGK) promoter was used to ubiquitously direct expression of histone-2B fused eGFP and mCherry proteins to the nucleus to monitor DNA content and enable tracking of cell migration and lineage. Vectors with T/Brachyury and alpha-myosin heavy chain (alphaMHC) promoters targeted fluorescent or drug-resistance proteins to early mesoderm and cardiomyocytes. The drug selection protocol yielded 96% pure cardiomyocytes that could be cultured for over 4 months. Puromycin-selected cardiomyocytes exhibited a gene expression profile similar to that of adult human cardiomyocytes and generated force and action potentials consistent with normal fetal cardiomyocytes, documenting these parameters in hESC-derived cardiomyocytes and validating that the selected cells retained normal differentiation and function. CONCLUSION/SIGNIFICANCE: The protocols, vectors and gene expression data comprise tools to enhance cardiomyocyte production for large-scale applications.

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